Shigeo MASUYAMA*, Kunio MITUI** & Narumi NAKATO***:

Studies on intraspecific polyploids of the fern *Lepisorus thunbergianus* (3)**** Mating system and the ploidy

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ノキシノブの種内倍数体の研究 (3) 受精様式と倍数性

In a comparative study on fern gametophytes, Masuyama (1975) reported two marked patterns as to the ontogenical sequence of gametangium formation: the pattern favorable for intergametophytic mating which is frequently found in diploid species, and the other for intragametophytic selfing frequently found in polyploid species. Later, Masuyama (1979, 1986) conducted mating experiments for the diploid and tetraploid strains of *Phegopteris decursive-pinnata* and showed that gametophytes of diploids were not successful in forming sporophytes by intragametophytic selfing because of the presence of genetic load, while those of tetraploids were quite free to form sporophytes by intragametophytic selfing. Besides these works, few studies have so far been carried out about the correlation between the mating system and the ploidy in ferns.

In the previous reports of this series (Nakato et al. 1983, Mitui et al. 1987), we documented the occurrence of 2n=50, 100, and 102 as main cytotypes in *Lepisorus thunbergianus* and showed their distributional patterns in Japan which are different to some extent from each other. In this report, the potential for intragametophytic selfing in the gametophytes of these strains will be treated to see whether such a positive correlation between the mating system and the ploidy as mentioned above is also the case with this species and, if so, whether the difference in mating system among these strains is relevant to their distributional patterns.

Materials and methods Spores were collected from four diploids (2n=50), four tetraploids (2n=100) and two hypertetraploids (2n=102) which were treated

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Tab. 1. Chromosome numbers and localities of the sporophytes examined.

Code of sporophyte	Chromosome no.	Locality
SY1	2n=50 (2X)	Yugashima, Shizuoka Pref.
SY2	2n=50 (2X)	Yugashima, Shizuoka Pref.
KO1	2n=50 (2X)	Ohyama, Kanagawa Pref.
KO2	2n=50 (2X)	Ohyama, Kanagawa Pref.
TK1	2n=100 (4X)	Okutama, Tokyo
TK2	2n=100 (4X)	Okutama, Tokyo
TI1	2n=100 (4X)	Okutama, Tokyo
TI2	2n=100 (4X)	Okutama, Tokyo
AI	2n=102 (4X')	Ikawa, Akita Pref.
AJ	2n=102 (4X')	Juniko, Aomori Pref.

in the previous studies (Tab. 1). Spores were sowed onto agar media containing inorganic nutrients in Petri dishes and cultured within a growth chamber (see Masuyama 1979 for the formula of media and the cultural condition). Two months after the spore sowing, 50 gametophytes were isolated from every spore source onto fresh media in tubes for an intragametophytic selfing test. As for SY1 and SY2, 20 sib paris (pair within SY1 gametophytes or SY2 gametophytes) and 20 non-sib pairs (pair between SY1 and SY2 gametophytes) were also inoculated into tubes for an intergametophytic mating test. The remaining gametophytes in dishes were kept in culture for one month more and then checked for the gametangial condition. After three weeks from the inoculation, isolated gametophytes were watered weekly and checked for the sporophyte formation. Waterings were carried out 15 times. As an additional experiment, isolated gametophytes of SY1 and SY2 were maintained in culture for eight months more after the last watering and then watered again six times at an interval of a month; that is, they were kept in culture for 1.5 year in total for this experiment. Besides these experiments, in order to see the sporophyte formation of gametophytes under rather natural environmental condition, spores obtained from three diploids and two tetraploids growing at Itsukaichi, Tokyo, were sowed on autoclaved soil in small clay pots in early summer. The pots were placed in a layer of water within a glass tank at the window, in which

the temperature was 7 to 28°C through the year. Some of the sporophytes obtained in these experiments were checked for the chromosome number in the following procedure: juvenile leaves were left in 8-hydroxyquinoline solution for 3 to 6 hours, fixed with a mixture of ethanol and acetic acid (3:1) for 20 to 60 minutes, and squashed in the same manner as given in the previous report (Nakato et al. 1983).

Results Tab. 2 shows the data suggesting the gametangial condition of gametophytes at the time when the mating experiments were started. As indicated by remarkably high proportions of females and functionally female hermaphrodites, gametophytes of all the spore sources formed antheridia at a low frequency and discontinuously at this time, though archegonia were formed frequently and constantly. Tab. 3 shows the antheridium formation of isolated gametophytes at the end of the selfing test, when all the gametophytes had branched repeatedly and established clones. Even 15 weeks after the first watering, the antheridium formation was not frequent; especially in the gametophytes of diploids (SY1 and SY2), 35 to 50% of them scarcely produced antheridia. About 1.5 year after, however, most of the gametophytes of these diploids had a number of mature antheridia on filamentous or prolonged portions of proliferations.

Tab. 2. Proportions of four sexual types in gametophyte populations cultured for three months.

Spore source	No. of samples	Vegetatives (%)	Males (%)	Females (%)	Hermaphrodites*			
					Total (%)	Occur. of MH	Occur. of FH	Occur. of RH
KO1 (2X)	50	0	0	66	34	−	###	−
KO2 (2X)	50	0	0	34	66	−	++	##
TK1 (4X)	50	6	0	12	82	−	###	−
TK2 (4X)	50	0	0	88	12	−	##	+
AI (4X')	50	0	0	62	38	−	###	−
AJ (4X')	50	0	0	46	54	−	##	+

* MH, functionally male hermaphrodites without mature archegonia; FH, functionally female hermaphrodites without mature antheridia; RH, real hermaphrodites with mature antheridia and archegonia. −, 0%; +, less than 25%; ++, 25 to 50%; ##, 51 to 75%; ###, more than 75%.

Tab. 3. Antheridium formation in isolated gametophytes at the end of the selfing test (15 weeks and 1.5 year after the first watering). C^-, clones without mature antheridia; C^+, clones with mature antheridia.

Spore source	15 weeks		1.5 year	
	No. C^-	No. C^+	No. C^-	No. C^+
SY1 (2X)	10 (50%)	10 (50%)	4 (8%)	46 (92%)
SY2 (2X)	7 (35%)	13 (65%)	5 (10%)	45 (90%)
TI1 (4X)	5 (25%)	15 (75%)	—	—
TI2 (4X)	3 (15%)	17 (85%)	—	—

Results of the intragametophytic selfing test are represented in Figs. 1 and 2. Most of the sporophytes produced by gametophytes of diploids retarded in appearance (Fig. 1) and were morphologically abnormal to be so small and often indented at the edge of leaf (Fig. 4). They seemed to have arisen apogamously because haploid chromosomes ($2n=25$) were observed on some of these sporophytes (Fig. 5). The occurrence frequency of normal sporophytes which might be formed sexually was as remarkably low as 4% at most (KO2). This trend was maintained throughout 1.5 year as in the cases of SY1 and SY2, during which additional waterings were done monthly. In contrast, gametophytes of tetraploids were much successful in intragametophytic selfing (Fig. 2); almost all the isolates formed normal sporophytes within 15 weeks. As for isolates of hypertetraploids, frequencies of sporophyte formation by selfing were 24% and 36%, being intermediate between those of diploids and tetraploids.

The results of intergametophytic mating tests are given in Fig. 3. In 20 sib pairs, 13% of SY1 gametophytes (5 gametophytes, no pair) and 5% of SY2 gametophytes (2 gametophytes, no pair) formed normal sporophytes. In 20 non-sib pairs between SY1 and SY2, 20% of gametophytes (8 gametophytes, one pair) formed normal sporophytes in the test.

In cultures on soil, whether diploids or tetraploids, cordate gametophytes occurred about a half year after the spore sowing. About one and a half year after the sowing, 28.6% and 39.5% of the gametophytes produced sporophytes in the cultures of two tetraploids, while only 0%, 1.4%, and 8.3% of the gameto-

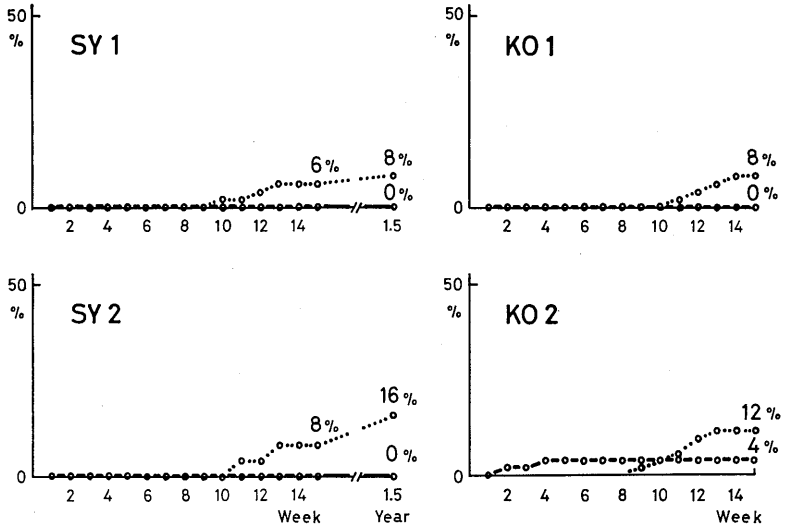

Fig. 1. Sporophyte formation in isolated gametophytes of diploids. Vertical and horizontal axes indicate the cumulative frequency of gametophytes forming sporophytes and the time after the first watering, respectively. Solid and dotted lines represent the gametophytes forming normal sporophytes and those forming abnormal ones, respectively.

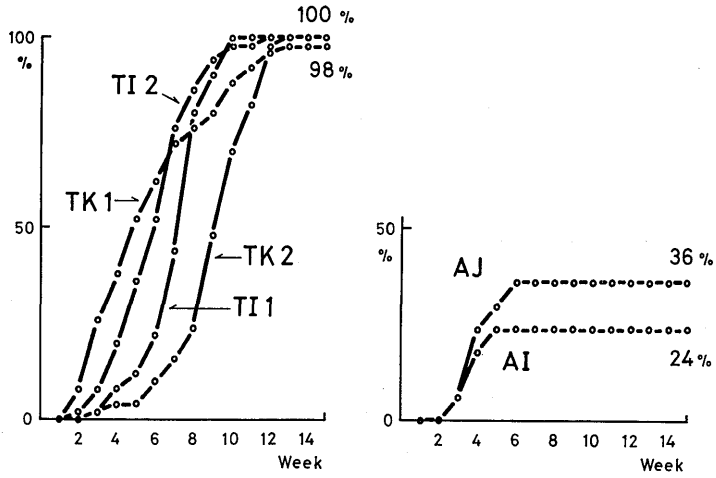

Fig. 2. Sporophyte formation in isolated gametophytes of tetraploids (TK1, TK2, TI1, and TI2) and hypertetraploids (AI and AJ). See Fig. 1 for explanation.

phytes in the cultures of three diploids (Fig. 6).

Discussion The most striking result obtained in this study is that isolated gametophytes of diploids of this species hardly formed sporophytes by selfing, but those of tetraploids did almost perfectly. Several causes are likely for this gap between diploids and tetraploids. The first to be supposed is the absence of antheridia on gametophytes of diploids through the course of the mating test. As suggested by the data in Tab. 2, gametophytes of this species, whether those of diploids or tetraploids, tended to form antheridia at low frequencies at the beginning of the mating test. In the gametophytes of diploids, this tendency was probably maintained to the end of the test (Tab. 3). However, it should be noted that they never formed sporophytes even 1.5 year after the first watering, when more than 90% of them had mature antheridia (Tab. 3). In addition, they formed sporophytes to some extent when not isolated but paired (Fig. 3). Therefore, it seems that the lack of sporophytes on isolated gametophytes of diploids is not attributable to the absence of antheridia on gametophytes.

Another possible cause is a genetic factor opposing to the sporophyte formation by intragametophytic selfing in diploids. The fact that almost all the isolated gametophytes of diploids did not form sporophytes throughout the mating test (Fig. 1) seems to indicate that diploids possess a genetic self-incompatibility

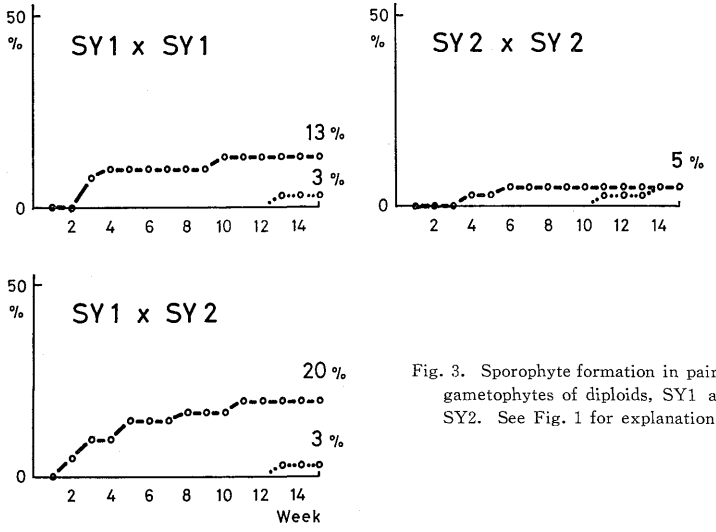

Fig. 3. Sporophyte formation in paired gametophytes of diploids, SY1 and SY2. See Fig. 1 for explanation.

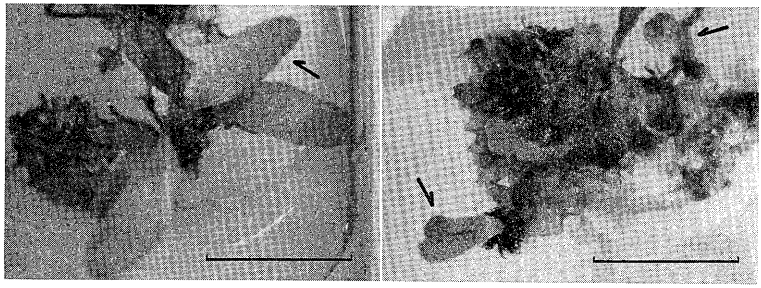

Fig. 4 Sporophytes (arrow) produced by isolated gametophytes of diploids. Left, normal sporophytes; right, abnormal ones. Scales, 1 cm.

system as reported by Wilkie (1956) in *Pteridium aquilinum*. However, as seen in Fig. 3, only 5% and 13% of the gametophytes formed sporophytes in sib pairs of SY1 and SY2 and also only 20% of the gametophytes in non-sib pairs between them. These values are remarkably low even if we assume that these two plants would be heterozygous for the same self-incompatible gene, for 50% of the gametophytes are expected to form sporophytes in that case. The genetic self-incompatibility system, therefore, is unlikely. The presence of genetic load, which was first reported for *Osmunda regalis* populations by Klekowski (1970) and have been revealed in many fern species (Klekowski 1984), is more likely as a genetic factor leading to the self-sterility in diploids of this species. If we assume, for example, that the diploids treated in this study would have been heterozygous for seven unlinked recessive lethal genes, the occurrence frequency of gametophytes forming sporophytes by intragametophytic selfing in isolated gametophytes is expected to be $(1/2)^7=0.008$ and that of gametophytes bearing sporophytes by intergametophytic selfing in sib paired gametophytes to be $(3/4)^7=0.133$, the values being similar to those obtained in this study (Figs. 1 and 3). However, if this assumption is valid, the fact that the occurrence frequency of gametophytes forming sporophytes was as low as 20% in the pairs between SY1 and SY2 (Fig. 3) would mean

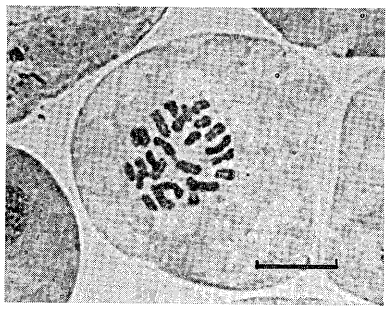

Fig. 5. Twenty-five haploid chromosomes in a somatic cell of an abnormal sporophyte. Scale, 10 μm.

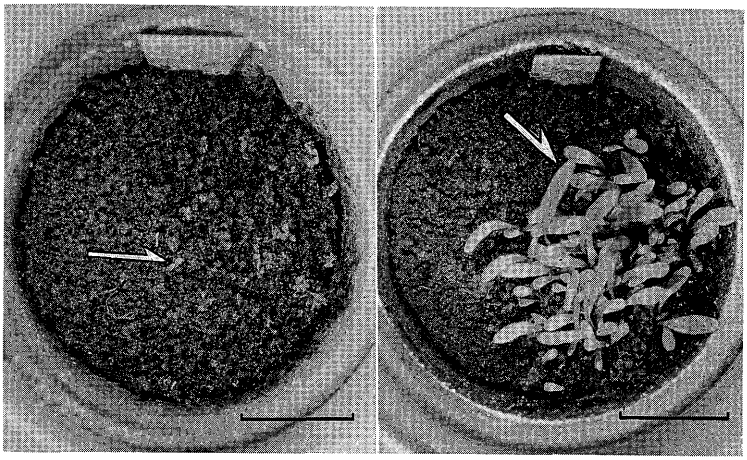

Fig. 6. Sporophytes (arrow) produced by gametophytes growing on soil, 20 months after the spore sowing. Left, diploid; right, tetraploid. Scales, 2 cm.

that these two plants may have possessed many identical lethal alleles. This seems to be rather improbable, because deleterious genes including lethal ones are generally considered to have arisen through mutation. The self-sterility in diploids, therefore, is not perfectly explicable in terms of the presence of genetic load.

Considering the epiphytic habit of this species, it may be possible to hypothesize that gametophytes of this species, especially those of diploids, need somewhat special physiological or ecological conditions to perform the fertilization and the embryogenesis. Evans & Bozzone (1977) and Evans & Conway (1980) reported that the viability of sperms and the success rate of sporophyte formation in gametophytes of *Pteridium aquilinum* were much dependent on pH of added water. DeMaggio (1963) also reported that embryos in gametophytes of *Todea barbara* were much affected by nutritional conditions on their development. Although available data have not been obtained in this study, it is suggested by these works that the above hypothesis is not unreasonable. The assumption of the presence of genetic load mentioned above and that of special physiological or ecological habit mentioned here are not alternatively but coordinately applicable for the account of the degree of selfing in this species. In diploids, sporophytes may be often heterozygous for many recessive lethal genes and also gametophytes may require special physiological and ecolog-

ical conditions for the fertilization and the embryogenesis. Gametophytes of diploids, therefore, are not usually successful in forming sporophytes by selfing and even by crossing. In tetraploids, on the other hand, sporophytes may be mostly free from genetic load as a result of frequent selfing and, even if they carry recessive lethals, duplicated loci may often block the expression of these lethals. Also gametophytes may be not so rigid as those of diploids in requiring special conditions for the fertilization and the embryogenesis. Gametophytes of tetraploids are thus quite successful in bearing sporophytes by selfing as well as crossing. These contrasted features on mating systems of diploids and tetraploids of this species probably occur not only in vitro but also in nature, as just seen in the gametophytes growing on soil (Fig. 6).

As for the degree of selfing in gametophytes of hypertetraploids, more substantial data may be needed before being discussed.

Masuyama (1975) recognized two types of gametangium formation in fern gametophytes; the non-simultaneous formation of antheridia and archegonia often found in diploid species and the simultaneous formation of these organs frequently found in polyploid species. However, gametophytes of *L. thunbergianus*, whether those of diploids or tetraploids, do not form antheridia easily and apt to proliferate readily, so that these clear patterns as to the ontogenical sequence of gametangium formation are not evident in this species. This seems to be related to the epiphytic habit of this species. Masuyama (1979, 1985) also reported that, in *Phegopteris decursive-pinnata*, gametophytes of diploids are genetically controlled to be rather unsuccessful in forming sporophytes by intragametophytic selfing while those of tetraploids are highly successful. A quite smilar situation is evident also in *L. thunbergianus*, as revealed in this study In the mating experiments with gametophytes of seven species in five genera, Verma & Bala (1979) noted that no correlation was recognized between the mating system and the ploidy in these species. However, unless based on the data for the polyploid taxa which are related to each other as closely as assorted into the same species or so, the definite conclusion could not be obtained as to whether a positive correlation is present between the ploidy and other specific characters such as the mating system.

Since intragametophytic selfing enables homosporous ferns to establish new sporophyte populations only by single spores (Baker 1955, Klekowski & Baker 1966), the degree of intragametophytic selfing may have an influence upon the

mode of distribution of ferns. In many works on the mating system of ferns, it has been revealed that intragametophytic selfing plays an important role in establishing disjunctive populations (Klekowski 1972, Cousens 1979, Crist & Farrer 1983, Watano 1986) and colonizing the open-habitats (Holbrook-Walker & Lloyd 1973, Lloyd 1974a, b). In this study, it should be pointed out that intragametophytic selfing may play a considerable role also in extending the range of distribution in ferns. As previously reported (Mitui et al. 1987), the tetraploids of *L. thunbergianus* which are widely distributed over Japan seem to have recently or even currently arisen directly from the diploids of this species which are distributed merely in southern half of Japan. Although the distributional expansion of the tetraploids toward the north may be basically owed to the wider tolerance of sporophytes for the cold climatic condition, it seems probable that the high potentiality for intragametophytic selfing in gametophytes of tetraploids as revealed in this study is also considerably responsible for the rapid expansion of distribution.

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S. C. & V. Bala 1979. *In* S. S. Bir (ed): Recent researches in plant sciences (Kalyani Pub., New Delhi), 237-243.

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ノキシノブの2倍体，4倍体，高4倍体の配偶体の自配受精能(単独で受精し，胞子体を形成する能力)を比較するために，受精実験を行い，次の結果を得た。自配受精率は4倍体(4株)ではほぼ100%，高4倍体(2株)では約30%前後であったが，2倍体(4株)は1株が4%であったのを除いて，あとは自配受精による胞子体形成が全く見られなかった。ただし，2倍体のばあい，株内かけ合わせと株間かけ合わせをさせると，5〜20%の配偶体が胞子体を形成した。2倍体と4倍体の自配受精率に大きな差をもたらす要因としていくつか考えられるが，2倍体のばあい，胞子体の遺伝的荷重が高く，かつ配偶体が受精と胚発生にあたって何らかの生理的，生態的に特殊な条件を必要とするのではないかと推定される。これに対し，4倍体は胞子体の遺伝的荷重が低く，配偶体も受精と胚発生にあたってそれほど特殊な条件を必要としないのではないかと推定される。2倍体と4倍体との間に自配受精能に大きな差があることを認めたのは，ゲジゲジシダ(益山1979, 1986)に次いで2度目である。このような差異は，2倍体が比較的限られた範囲に分布し，4倍体が比較的広範囲に分布することとも関連しているものと思われる。

□たくぎん総合研究所（伊藤浩司・日野間彰 編著）：**環境アセスメントのための北海道高等植物目録IV 合弁花植物** 244 pp. 1987. たくぎん総合研究所（札幌市中央区大通西3-6 道新ビル）. ¥7,000. 1985年にIとしてシダ植物・裸子植物が刊行されている。電算機により学名，和名を整理して示してあり，この種のデータ処理のむずかしさを知る者として最大限の敬意を表したい。産地は支庁単位である。巻末にデータベースからの出力例として，ラインプリンタによる分布図が示されており，この目録に表示された以外の詳細なデータが蓄積されているようであるので，ノウハウが披露されれば，他地域での仕事に有効な手本となるだろう。続刊を期待する。 (金井弘夫)

□志村義雄：**富士山のシダ** 132 pp. 1987. 静岡新聞社，静岡. ¥3,000. 静岡県の植物特にシダを長年研究している著者が，わが庭木のように可愛がっている富士山のシダをまとめたもの。自生のシダ213種と38雑種の目録に，各種ごとの産地・分布などの記事があるほか，別項で分類上・分布上・生態上興味ある種についての解説がある。標本および生態の写真90個があってわかりやすい。その他富士山のシダの垂直分布，南面と北面の比較，文献など。特産のスルガイノデは今までアスカイノデの変種とされていたが，今回種に上げられた。発売元は静岡市登呂3-1-1 静岡新聞社出版局。 (伊藤 洋)